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Biofilm formation and autoinducer-2 signaling in *Streptococcus intermedius*: role of thermal and pH factors

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Background/aim: Bacteria sense their population density using autoinducer (AI) signaling systems. The AI-2 signal is thought to mediate communication among and within bacterial species. *Streptococcus intermedius* is a commensal organism frequently associated with periodontitis and purulent infections. We investigated the role of AI-2 signaling in *S. intermedius* biofilm formation under temperatures and pH levels relevant to human physiology.
Methods: Bioluminescence was used to monitor the change in AI-2 levels at various temperatures. Growth and biofilm formation in *S. intermedius* and its *luxS* mutant SI006 were measured at 35, 37, 39, and 41°C and in pH ranging from 5.7 to 7.5. To confirm the role of AI-2 signals in biofilm formation, the AI-2 precursor (S)-4,5-dihydroxy-2,3-pentanedione (DPD) was used to complement SI006 biofilm formation.
Results: *S. intermedius* AI-2 signals were detected at all growth temperatures but reached the highest levels at 37°C. SI006 displayed significantly less biofilm formation than *S. intermedius* wild-type (WT); however, the role of AI-2 on biofilm formation was confined to 37°C. DPD supplementation significantly increased SI006 biofilm formation to the *S. intermedius* WT level. The role of AI-2 in *S. intermedius* biofilm formation was not influenced by pH. High temperatures and low pH enhanced biofilm formation in both *S. intermedius* and its *luxS* mutant.
Conclusions: High temperature and acidic conditions may favor biofilm formation by *S. intermedius*. The role of AI-2 in biofilm formation by *S. intermedius*, however, varies with physiological temperature changes. These results may assist in understanding possible behavior relative to health and disease.

Key words: autoinducer-2; biofilm; *luxS*; pH; *Streptococcus intermedius*; temperature

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The human body comprises several dynamic microenvironments in health and disease. The oral cavity, for example, may constitute a wide variety of niches that favor distinct microbial habitats. Furthermore, the ecological shifts during disease provide a competitive advantage for colonization of several opportunistic microorganisms. Despite individual variations, plaque pH varies from 5.6 to 6.5 while the pH of resting saliva is approximately 6.7 (12, 15). The pH values may also differ depending on the location of the tooth, the tooth surface, and diet (12). Purulent infections and dental caries usually create acidic environments (6, 20). The pH value in subgingival regions becomes alkaline at the onset of periodontal disease, favoring gram-negative bacteria (2). The human body maintains a steady core temperature of approximately 37°C (36.8 ± 0.4) during health (10). In the oral cavity, temperatures vary by location and tissue (27). Localized temperature increases have also been

observed during inflammation and progression of periodontal disease (13). Changes in temperature leading to fever (>37.4°C), hyperpyrexia (≥39°C) or even hypopyrexia (<36°C) may accompany infections resulting in sepsis and even a fatal outcome (7).

Existing as multitudes of interacting species, bacteria can take advantage of their growing populations to initiate chemical dialog, referred to as quorum sensing. Quorum sensing allows bacteria to sense each other and their environment via autoinducers (AI). At a critical concentration of AI, the bacterial quorum initiates cascades of gene regulation conducive to adaptation and survival. The presence of type 2 autoinducers (AI-2) and their enzymatic synthase LuxS in several gram-positive and gram-negative species suggest that AI-2 are comprehensive bacterial communication signals. LuxS catalyzes the synthesis of the AI-2 precursor (S)-4,5-dihydroxy-2,3-pentanedione (DPD) through the activated methyl cycle (29). DPD subsequently reorganizes into several derivatives, collectively termed AI-2. AI-2 signaling has been associated with the coordination of several virulent behaviors in bacteria, including the environmental stress response, single-species/dual-species biofilm formation, and toxin expression (11, 17, 19, 24, 26, 32). Biofilms play an important role in several chronic infections because of their ability to challenge the host immune system and resist antimicrobial treatment (3). In the oral cavity, biofilms may cause dental caries, periodontitis, and endodontic infections (3, 25).

Streptococcus intermedius, *Streptococcus anginosus*, and *Streptococcus constellatus*, collectively known as the *S. anginosus* group, are members of the normal microflora of the mouth and throat. The *S. anginosus* group may also be involved in life-threatening purulent infections (28). In the oral cavity, acute third molar pericoronitis (16) and periapical abscesses are frequently associated with the *S. anginosus* group (8). *S. intermedius* is frequently associated with chronic periodontitis and upper-body abscesses, especially brain abscesses (22, 30).

The aim of this study was to investigate the role of AI-2 in *S. intermedius* biofilm formation under various physiological temperatures and pH environments. We found that extracellular AI-2 levels were temperature dependent in *S. intermedius*, reaching optimum values at 37°C. The role of *luxS*/AI-2 in biofilm formation was limited to 37°C independent of pH values.

Materials and methods

Bacterial strains and media

The *luxS* gene in *S. intermedius* NCTC 11324 was disrupted as described previously (17). *S. intermedius* 11324 wild-type (WT) and the resultant *luxS* isogenic mutant SI006 were stored at −20°C. The *S. intermedius* strains were first cultured on Todd–Hewitt agar plates (Difco Laboratories, Detroit, MI) for 24 h at 37°C in 5% CO_2 in air. Before each experiment, bacteria were cultured overnight in tryptone soy broth (TSB, Oxoid, Hampshire, UK). SI006 was supplied with 0.5 μg/ml kanamycin (Sigma-Aldrich, St Louis, MO) during the first overnight growth.

Vibrio harveyi BB 170, the AI-2 reporter, was grown on heart infusion agar or in heart infusion medium supplemented with 0.1 μg/ml kanamycin at 30°C in air with shaking. Second overnight cultures were grown in the autoinducer bioassay medium as previously described (24). *V. harveyi* BB 170 was generously donated by Prof. B. Bassler.

AI-2 bioluminescence induction

To examine the effect of various physiological temperatures on AI-2 signaling in *S. intermedius*, a *V. harveyi* bioluminescence assay was used to compare AI-2 levels as described by Surette and Bassler (24). The *S. intermedius* WT was grown in TSB adjusted to pH 7.0 with 0.2 M phosphate buffer and incubated at 35, 37, or 39°C (±0.04°C) in microaerophilic atmosphere (Mart® Microbiology Anoxomat-System version 1, Drachten, the Netherlands). The bacterial cultures maintained relative stable pH values for up to 8 h. The pH values did not vary with incubation temperatures and dropped approximately one unit by stationary phase. Bacterial cultures collected after 2 to 24 h of growth, were centrifuged at 16438 × ***g*** and then filtered through 0.22-μm pore filters. Frozen *V. harveyi* cultures diluted 1 : 500 in fresh autoinducer bioassay medium were prepared as previously described (19). Filtered *S. intermedius* supernatants were added to *V. harveyi* BB 170 (1 : 10) in flat-bottomed 96-well microtiter plates. *V. harveyi* supernatants and DPD (2 μM to 0.4 mM) were used as positive controls. AI-2 induction in *V. harveyi* BB 170 with TSB was used as background. AI-2-induced bioluminescence from *S. intermedius* supernatants was calculated relative to 4 μM DPD supplementation (=100%). Bioluminescence was measured using KC4™ V 3.4 (Bio-Tek® Instruments, Inc., Winooski, VT).

Biofilm formation and planktonic growth

To investigate the role of AI-2 in *S. intermedius* biofilm formation, sterile Thermanox™ plastic coverslips (Nunc, Copenhagen, Denmark) were inserted into 24-well microtiter plates. Overnight bacterial cultures diluted in TSB (1 ml, 1%) were dispensed into the microtiter plates and left overnight at 37°C under aerobic conditions in 5% CO_2. Initial biofilms (20 h) were additionally supplemented with fresh TSB (0.5 ml) and further incubated (18 h) to allow for coherent biofilm formation. The biofilm-covered disks were then rinsed with water, stained with 0.1% Safranin, and rinsed in water again before the addition of 30% acetic acid. The released Safranin was measured at 530 nm in the KC4™ V 3.4. Total growth of *S. intermedius* in TSB was evaluated in parallel microtiter plates and optical density was measured at 595 nm (OD_{595}) in the KC4™ V 3.4.

To investigate whether SI006 biofilm formation could be complemented by AI-2 precursor, biofilms were formed in TSB supplemented with DPD (Omm Scientific Inc., Dallas, TX). To determine specific complementation concentrations for SI006, DPD was assayed at a range of 0.08–80 nM as described for other streptococci (19).

To measure biofilm formation under varying temperatures and pH gradients, bacterial cultures were inoculated in TSB buffered with phosphate buffer to final pH values of 5.7, 6.5, 7.0, or 7.5. The microtiter plates were incubated at 35, 37, 39, or 41°C (±0.04°C) under microaerophilic conditions. The experiment was repeated four times with three parallels.

The planktonic growth of *S. intermedius* WT and SI006 incubated at 35, 37, 39, or 41°C (±0.04°C) and at various pH values (pH 5.7–7.5) was measured at 2, 4, 6, 8, and 18 h using a KC4™ V 3.4 spectrophotometer at 595 nm.

Statistical analysis

The Student's *t*-test or one-way analysis of variance followed by the Student–Newman–Keuls method were used for comparisons. Differences were considered statistically significant at $P \leq 0.05$.

Results

S. intermedius and SI006 displayed similar planktonic growth rates

The planktonic growth of *S. intermedius* WT and SI006 was similar irrespective of incubation temperatures and pH values (Table 1). Incubation temperatures >35°C accelerated the growth rate of *S. intermedius* after 5 h incubation (Fig. 1A).

AI-2 levels in *S. intermedius* supernatants vary with temperature

Bacteria live at varying physiological temperatures, so we investigated whether AI-2 signaling was influenced by temperature. Bioluminescence induction in *V. harveyi* BB 170 indicated that *S. intermedius* produced AI-2 molecules at all the temperatures tested and that AI-2 levels in *S. intermedius* supernatants varied with incubation temperature (Fig. 1B). AI-2 levels were highest at 37°C, reaching maximum values at early exponential growth phase (5–6 h growth, OD_{595} 0.1–0.2) (Fig. 1A,B). AI-2 induction in supernatants collected from 39°C cultures was lower than at 37°C and declined at early exponential phase. Compared to other temperatures, supernatants collected from 35°C cultures displayed the lowest AI-2 values, delayed AI-2 induction and decline. At all temperatures, AI-2 levels dropped to background levels when *S. intermedius* reached stationary growth phase.

Table 1. Growth of *Streptococcus intermedius* wild-type (WT) and SI006 measured as optical density at 595 nm (standard error of the mean) at 8 and 18 h under various temperature and pH values

	8 h	18 h
35°C		
WT		
pH 7.5	0.10 (0.003)	0.37 (0.011)
pH 7	0.09 (0.003)	0.56 (0.012)
pH 6.5	0.08 (0.002)	0.53 (0.013)
pH 5.7	0.06 (0.001)	0.44 (0.008)
SI006		
pH 7.5	0.10 (0.020)	0.38 (0.013)
pH 7	0.09 (0.007)	0.55 (0.004)
pH 6.5	0.09 (0.005)	0.53 (0.007)
pH 5.7	0.06 (0.001)	0.43 (0.007)
37°C		
WT		
pH 7.5	0.27 (0.007)	0.45 (0.003)
pH 7	0.31 (0.003)	0.52 (0.003)
pH 6.5	0.34 (0.002)	0.55 (0.003)
pH 5.7	0.12 (0.003)	0.45 (0.008)
SI006		
pH 7.5	0.26 (0.009)	0.45 (0.004)
pH 7	0.30 (0.007)	0.52 (0.002)
pH 6.5	0.33 (0.005)	0.56 (0.003)
pH 5.7	0.12 (0.006)	0.44 (0.015)
39°C		
WT		
pH 7.5	0.33 (0.005)	0.44 (0.014)
pH 7	0.38 (0.008)	0.55 (0.009)
pH 6.5	0.40 (0.002)	0.56 (0.019)
pH 5.7	0.17 (0.003)	0.42 (0.007)
SI006		
pH 7.5	0.32 (0.006)	0.42 (0.004)
pH 7	0.37 (0.023)	0.54 (0.012)
pH 6.5	0.39 (0.008)	0.57 (0.005)
pH 5.7	0.16 (0.002)	0.40 (0.013)
41°C		
WT		
pH 7.5	0.40 (0.003)	0.47 (0.004)
pH 7	0.46 (0.009)	0.53 (0.003)
pH 6.5	0.48 (0.009)	0.58 (0.007)
pH 5.7	0.27 (0.004)	0.50 (0.006)
SI006		
pH 7.5	0.41 (0.006)	0.44 (0.004)
pH 7	0.47 (0.005)	0.53 (0.003)
pH 6.5	0.47 (0.003)	0.59 (0.004)
pH 5.7	0.24 (0.021)	0.49 (0.007)

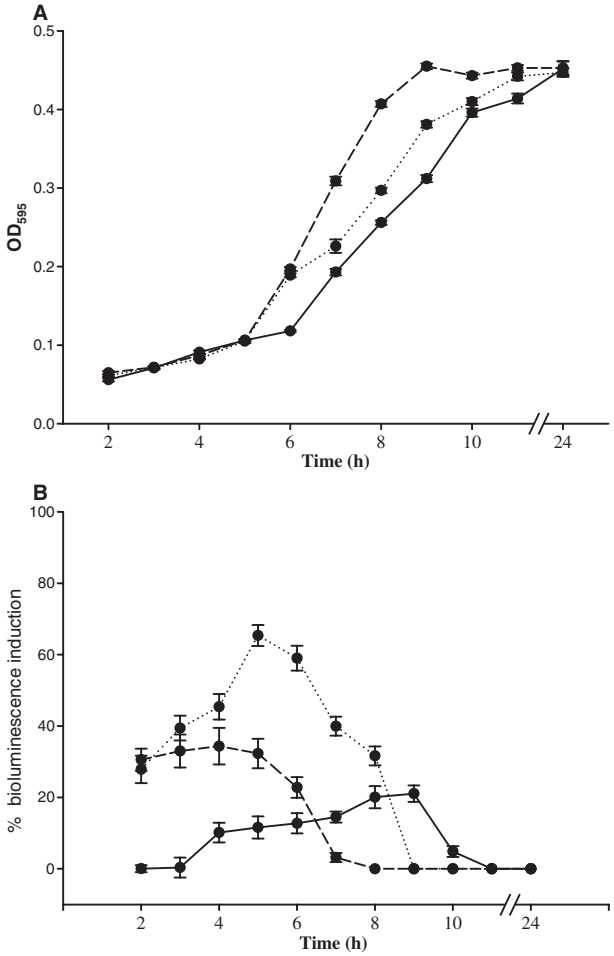

Fig. 1. The influence of temperature on *Streptococcus intermedius* growth and autoinducer-2 (AI-2) levels. (A) Growth of *S. intermedius* at 39°C (interrupted line), 37°C (dotted line), and 35°C (solid line). Mean values ($n = 9$) with standard error of the mean are given. (B) *S. intermedius* wild-type (WT) bioluminescence induction during growth. The *S. intermedius* WT supernatants incubated at 35°C (solid line), 37°C (dotted line), or 39°C (interrupted line) were collected throughout growth (2–24 h). AI-2 induction is displayed relative to 4 μM (S)-4,5-dihydroxy-2,3-pentanedione (DPD; 100%). Data points represent mean values ($n = 9$) with standard error of the mean.

S. intermedius biofilm formation is influenced by AI-2 signaling, temperature, and pH values

We initially investigated whether AI-2 was involved in biofilm formation by *S. intermedius*. The *luxS* mutant SI006 formed 29% less biofilm compared to *S. intermedius* WT ($P \leq 0.05$, Fig. 2A). Scraping and resuspension of the biofilms in 1 ml TSB showed similar reduction from 0.58 (±0.011) in *S. intermedius* WT to 0.4 (±0.0123) in SI006 at OD_{600}. DPD displayed the highest complementation at 0.8–8 nM (data not shown). Upon delivery of 0.8 nM DPD, biofilm formation by SI006 increased by 21% (Fig. 2A), while DPD had no apparent effect on *S. intermedius* WT growth nor on biofilm formation (Fig. 2).

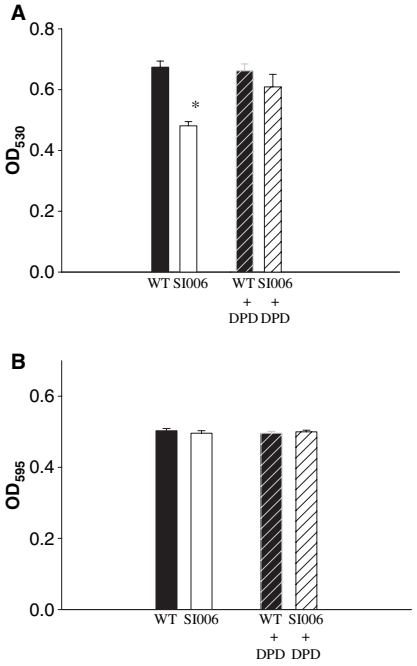

Fig. 2. Streptococcus intermedius and (S)-4,5-dihydroxy-2,3-pentanedione (DPD) supplementation (A) Biofilm formation in *S. intermedius* wild-type (WT) (black bars) and SI006 *luxS* mutant (white bar) without (plain bars) and with (striped bars) 0.8 nM DPD. Vertical bars represent mean values ($n = 9$) with the standard error of the mean. (B) Total growth in *S. intermedius* WT (black bars) and SI006 *luxS* mutant (white bar) without (plain bars) and with (striped bars) 0.8 nM DPD. Vertical bars represent mean values ($n = 9$) with the standard error of the mean. Asterisks indicate significantly less than *S. intermedius* WT or SI006 supplied with DPD.

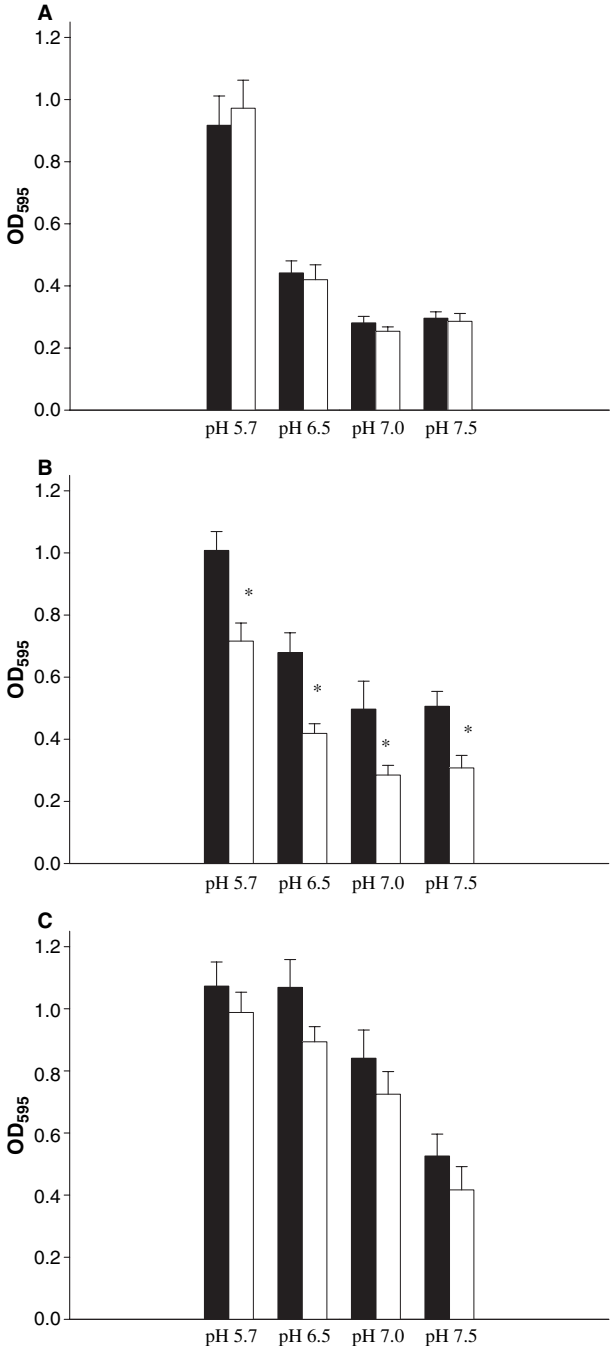

Fig. 3. Biofilm formation in *Streptococcus intermedius* wild-type (WT) (black bars) and SI006 *luxS* mutant (white bars) at (A) 35°C, (B) 37°C, and (C) 39°C. Biofilms were formed at pH 7.5, 7.0, 6.5, and 5.7. Vertical bars represent mean values ($n = 9$) with the standard error of the mean. Asterisk indicates significantly less than the respective *S. intermedius* WT.

Since temperature affected the pattern of *S. intermedius* AI-2 levels, our next step was to investigate whether temperature may also influence biofilm formation by *S. intermedius* at different pH levels. Our results showed that at 37°C, biofilm formation by SI006 was 39, 43, 38, and 29% less than that by *S. intermedius* WT at pH 7.5, 7.0, 6.5, and 5.7, respectively ($P \leq 0.05$) (Fig. 3B). However, at 35 and 39°C, biofilm formation by SI006 was not significantly different from that by *S. intermedius* WT (Fig. 3A,C). At 41°C, biofilm formation in *S. intermedius* WT and SI006 was similar to that at 39°C (data not shown).

Both *S. intermedius* and its *luxS* mutant SI006 exhibited significant biofilm formation when incubated at higher temperatures and acidic conditions, reaching optimum biofilm formation when simultaneously exposed to 39°C and an acidic environment (pH 5.7–6.5) (Fig. 3C). At all temperatures tested, biofilm formation was two to three times higher at pH 5.7 than at pH 7.5 (Fig. 3A–C). At pH levels 7.5, 7.0, and 6.5, the biofilm mass increased approximately 1.6 times when temperatures increased from 35 to 37°C and 1.6 and two times at pH 7 and pH 6.5 when temperatures were raised from 37 to 39°C (Fig. 3A–C).

Discussion

In the current study, we showed that the influence of AI-2 on biofilm formation in *S. intermedius* depended on temperatures between 35 and 39°C, but not on pH. We observed an association between the effect on biofilm formation and extracellular AI-2 levels in *S. intermedius*. AI-2 reached

maximum levels at 37°C followed by 39°C and 35°C, respectively. Interestingly, a temperature rise from 35°C to 39°C and low pH (5.7–6.5) significantly enhanced biofilm mass in *S. intermedius* WT and the *luxS* mutant.

The influence of AI-2 on biofilm formation has been reported in various species, including streptococci (17, 19, 26). In most studies the inferred role is based on results showing a defective ability of *luxS* mutants to form biofilms. However, because LuxS possesses an inherent metabolic function in the activated methyl cycle, phenotypic defects in *luxS* mutants may not strictly be attributed to AI-2 signaling but possibly to metabolic disturbances. For instance, biofilm defects in a *Lactobacillus rhamnosus luxS* mutant are not restored by AI-2 molecules but rather by the addition of cysteine, indicating a sole metabolic role of LuxS (14). Complementation studies may involve the addition of AI-2-positive supernatants from wild-type strains (31). However, *luxS* inactivation may disturb several integral processes within the activated methyl cycle. Therefore, supernatant supplementation of *luxS* mutants may complement other deficiencies in addition to AI-2 molecules. More recently, the availability of synthetic AI-2 precursors (DPD) has permitted specific AI-2 complementation and concentration comparisons with other bacterial species. In our study, DPD restored biofilm formation by the *S. intermedius luxS* mutant at a specific concentration range similar to that of *Streptococcus oralis luxS* mutants (19). However, DPD concentrations required for bioluminescence induction in *V. harveyi* were higher than those required for biofilm formation in *S. intermedius*. Variation in DPD concentrations may reflect the influence of different DPD thresholds on various bacterial functions. The concentration-dependent DPD restoration of defective *luxS* biofilms suggests that AI-2 molecules may function as signals and not merely as metabolic by-products in *S. intermedius*.

AI-2 signaling may differ with bacterial species, growth phase, and a variety of environmental factors including glucose availability, osmolarity, and acidity (17, 24). In *Salmonella typhimurium*, AI-2 production ceases under intense thermal shock (24). Though *S. intermedius* produced AI-2 at all temperatures, higher values were reached at 37°C. The role of *luxS* in biofilm formation at this temperature may reflect the significance of attaining threshold AI-2 levels in *S. intermedius*. In previous studies, we displayed the abundance of AI-2 production in the early growth phase of most oral streptococci (17). Our current results may imply that temperature-induced modifications in extracellular AI-2 levels may alter bacterial behavior during febrile infections.

Under all the pH levels assayed, *S. intermedius luxS* mutation resulted in deficient biofilm formation at 37°C, indicating that pH fluctuation was not a determining factor in AI-2-associated biofilm formation. In contrast, low pH induced the autoinducer synthesis protein LuxS in *Escherichia coli* and *Lactococcus lactis* (9, 23). However, because pH is a confounding factor in the *V. harveyi* BB 170 luminescence induction assay (5), measurement of AI-2 levels at various pH values was avoided in the current study.

The human body contains a wide array of micromilieus during health and disease. Host environments may vary for instance in oxygen tension, temperature, pH, and nutrient availability. For most streptococci, growth is either reduced or inhibited at temperatures above 37°C (21, 26). Surprisingly, we found that high temperatures accelerated growth and biofilm formation by *S. intermedius*. Infections and inflammation are commonly accompanied by systemic and localized temperature rise so the ability of *S. intermedius* biofilms to thrive at high temperatures may contribute to its role in periodontitis and febrile suppurative infections. Temperature changes may elicit a range of responses in bacterial biofilms. For instance, a temperature rise from 37 to 40°C suppresses biofilm formation in *Streptococcus mutans* (26). In *Porphyromonas gingivalis*, temperature increase above 37°C causes significant reduction in adhesion because of the downregulation of fimbrial expression (1) while thermal stress induces polysaccharide intercellular adhesin expression in *Staphylococcus epidermidis* (18). In *Streptococcus pyogenes*, microarray investigation identified specific gene regulation patterns that occurred only when exposed to both high temperatures (40°C) and acidic environments (pH 6) (4). In our study, *S. intermedius* biofilm formation reached peak values when exposed to 39°C and acidic environments (pH 5.7–6.5) simultaneously. Therefore, because abscesses occur in low pH environments (20), infections may favor the establishment of *S. intermedius* biofilms.

We conclude that in *S. intermedius*, AI-2 levels and its role in biofilm formation varied with temperature. Bacteria are both symbiotic and pathogenic residents of the human host. Under equilibrium and in health, it may be convenient for commensal species to adapt to normal host temperatures and to exist in harmony with the other cohabitants. *S. intermedius* is part of the normal bacterial flora and therefore may employ AI-2 signaling to its maximum level when collaboration is necessary. Under pathogenic conditions associated with thermal changes, AI-2 levels may not be as relevant.

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References

1. Amano A, Premaraj T, Kuboniwa M et al. Altered antigenicity in periodontitis patients and decreased adhesion of *Porphyromonas gingivalis* by environmental temperature stress. Oral Microbiol Immunol 2001: **16**: 124–128.
2. Bickel M, Cimasoni G. The pH of human crevicular fluid measured by a new microanalytical technique. J Periodontal Res 1985: **20**: 35–40.
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999: **284**: 1318–1322.
4. Dalton TL, Collins JT, Barnett TC, Scott JR. RscA, a member of the MDR1 family of transporters, is repressed by CovR and required for growth of *Streptococcus pyogenes* under heat stress. J Bacteriol 2006: **188**: 77–85.
5. De Keersmaecker SC, Vanderleyden J. Constraints on detection of autoinducer-2 (AI-2) signalling molecules using *Vibrio harveyi* as a reporter. Microbiology 2003: **149**: 1953–1956.
6. Dirksen TR, Little MF, Bibby BG, Crump SL. The pH of carious cavities. I. The effect of glucose and phosphate buffer on cavity pH. Arch Oral Biol 1962: **7**: 49–57.
7. Elebute EA, Stoner HB. The grading of sepsis. Br J Surg 1983: **70**: 29–31.
8. Fisher LE, Russell RR. The isolation and characterization of milleri group streptococci from dental periapical abscesses. J Dent Res 1993: **72**: 1191–1193.
9. Frees D, Vogensen FK, Ingmer H. Identification of proteins induced at low pH in *Lactococcus lactis*. Int J Food Microbiol 2003: **87**: 293–300.
10. Gelfand JA, Dinarello CA, Wolff SM. Harrison's principles of internal medicine, 13th edn. New York: McGraw-Hill, 1994: 81–89.
11. Kim SY, Lee SE, Kim YR et al. Regulation of *Vibrio vulnificus* virulence by the LuxS

quorum-sensing system. Mol Microbiol 2003: **48**: 1647–1664.
12. Kleinberg I. Formation and accumulation of acid on the tooth surface. J Dent Res 1970: **49**: 1300–1317.
13. Kung RT, Ochs B, Goodson JM. Temperature as a periodontal diagnostic. J Clin Periodontol 1990: **17**: 557–563.
14. Lebeer S, Verhoeven TL, Perea Velez M, Vanderleyden J, De Keersmaecker SC. Impact of environmental and genetic factors on biofilm formation by the probiotic strain *Lactobacillus rhamnosus* GG. Appl Environ Microbiol 2007: **73**: 6768–6775.
15. Moreno EC, Margolis HC. Composition of human plaque fluid. J Dent Res 1988: **67**: 1181–1189.
16. Peltroche-Llacsahuanga H, Reichhart E, Schmitt W, Lutticken R, Haase G. Investigation of infectious organisms causing pericoronitis of the mandibular third molar. J Oral Maxillofac Surg 2000: **58**: 611–616.
17. Petersen FC, Ahmed NA, Naemi A, Scheie AA. LuxS-mediated signalling in *Streptococcus anginosus* and its role in biofilm formation. Antonie Van Leeuwenhoek 2006: **90**: 109–121.
18. Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrob Agents Chemother 2000: **44**: 3357–3363.
19. Rickard AH, Palmer RJ Jr, Blehert DS et al. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. Mol Microbiol 2006: **60**: 1446–1456.
20. Simmen HP, Battaglia H, Giovanoli P, Blaser J. Analysis of pH, pO_2 and pCO_2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. Infection 1994: **22**: 386–389.
21. Small PM, Tauber MG, Hackbarth CJ, Sande MA. Influence of body temperature on bacterial growth rates in experimental pneumococcal meningitis in rabbits. Infect Immun 1986: **52**: 484–487.
22. Socransky SS, Haffajee AD, Dzink JL. Relationship of subgingival microbial complexes to clinical features at the sampled sites. J Clin Periodontol 1988: **15**: 440–444.
23. Stancik LM, Stancik DM, Schmidt B, Barnhart DM, Yoncheva YN, Slonczewski JL. pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*. J Bacteriol 2002: **184**: 4246–4258.
24. Surette MG, Bassler BL. Regulation of autoinducer production in *Salmonella typhimurium*. Mol Microbiol 1999: **31**: 585–595.
25. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endodontic Topics 2004: **9**: 27–36.
26. Tam K, Kinsinger N, Ayala P, Qi F, Shi W, Myung NV. Real-time monitoring of *Streptococcus mutans* biofilm formation using a quartz crystal microbalance. Caries Res 2007: **41**: 474–483.
27. Volchansky A, Cleaton-Jones P. Variations in oral temperature. J Oral Rehabil 1994: **21**: 605–611.
28. Whiley RA, Beighton D, Winstanley TG, Fraser HY, Hardie JM. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. J Clin Microbiol 1992: **30**: 243–244.
29. Winzer K, Hardie KR, Burgess N et al. LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(^{2}H)-furanone. Microbiology 2002: **148**: 909–922.
30. Yamamoto M, Fukushima T, Ohshiro S et al. Brain abscess caused by *Streptococcus intermedius*: two case reports. Surg Neurol 1999: **51**: 219–222.
31. Yoshida A, Ansai T, Takehara T, Kuramitsu HK. LuxS-based signaling affects *Streptococcus mutans* biofilm formation. Appl Environ Microbiol 2005: **71**: 2372–2380.
32. Yuan L, Hillman JD, Progulske-Fox A. Microarray analysis of quorum-sensing-regulated genes in *Porphyromonas gingivalis*. Infect Immun 2005: **73**: 4146–4154.